CLAIMS

1. A process for production of recombinant arylsulphatase A in a continuous cell culture system, the process comprising:

i) culturing a mammalian cell capable of producing arylsulfatase A in liquid medium in a system comprising one or more bio-reactors;

ii) concentrating, purifying and formulating the rhASA by a purification process comprising one or more steps of affinity chromatography and/or ion exchange chromatography.

2. A process according to claim 1, wherein said mammalian cell comprises a nucleic acid sequence, which encodes:

(a) the amino acid sequence of SEQ ID NO:2;

(b) a portion of the sequence in (a), which is enzymatically equivalent to recombinant human arylsulfatase A

(c) an amino acid sequence analogue having at least 75% sequence identity to any one of the sequences in (a) or (b) and at the same time comprising an amino acid sequence, which is enzymatically equivalent to recombinant human arylsulfatase A.

3. A process according to any of the preceding claims, wherein the production of arylsulfatase A or its equivalent occurs at a rate and under conditions which result in a product comprising a glycoform of the enzyme having four glycosylation intermediates as determined by MALDI-TOF analysis after treatment with low concentrations of PNGase F.

4. A process according to any of the preceding claims, wherein the carbohydrate moieties of the arylsulfatase A or its equivalent have a combined mass of 3-8 kDa.

5. A process according to any the preceding claims, wherein the production of arylsulfatase A or its equivalent occurs at a rate and under conditions which result in a product comprising a glycoform of the enzyme having a pattern of high mannose and/or complex oligosaccharides, which are phosphorylated so as to allow efficient endocytosis of the enzyme via mannose-6-phosphate receptor mediated entry.

6. A process according to any of the preceding claims, wherein production of the arylsulfatase A or its equivalent occurs at a rate and under conditions, which result in a product comprising an isoform of the enzyme in which the amino acid corresponding to Cys-69 in SEQ ID NO: 2 is converted to Formylglycine, corresponding to Fgly-51 in SEQ ID NO: 3.

7. A process according to any of the preceding claims, wherein the arylsulfatase A produced is selected from the group consisting of

(a) the amino acid sequence of SEQ ID NO:3;

(b) a portion of the sequence in (a), which is enzymatically equivalent to recombinant human arylsulfatase A

(c) an amino acid sequence analogue having at least 75% sequence identity to any one of the sequences in (a) or (b) and at the same time being enzymatically equivalent to recombinant human arylsulfatase A.

8. A process according to any of the preceding claims, wherein the mammalian cells are of human or primate origin.

9. A process according to any of the preceding claims, wherein the concentration and purification process of ii) comprises one or more steps of Expanded Bed Chromatography.

10. A process according to any of the preceding claims, wherein the concentration and purification process of (ii) comprises a polishing step including a passive step, wherein the arylsulfatase A passes through an affinity chromatography resin or membrane and/or a cation chromatography resin or membrane, and an active step, wherein the arylsulfatase A is detained within and subsequently eluted from an anion exchange membrane or resin.

11. A process according to any of the preceding claims, wherein the concentration and purification process of ii) comprises the following steps:

II) contacting an arylsulfatase A containing supernatant on an equilibrated chromatography column and eluting one or more fraction(s) containing arylsulfatase A;

III) loading the fraction(s) from step II on another equilibrated chromatography column and eluting one or more fraction(s) containing arylsulfatase A;

IV) buffer exchange of the arylsulfatase A present in the fraction(s) from step III by tangential flow filtration;

V) polishing the preparation of arylsulfatase A from step IV in one or two or more successive steps, each step comprising loading the preparation on an equilibrated chromatography columns and eluting one or more fraction(s) containing arylsulfatase A;

VI) passing the fraction(s) from step V through a viral reduction filter;

VII) formulating the fraction(s) from step VI in order to obtain a preparation of arylsulfatase A in a suitable formulation buffer;

VIII) optionally filling the formulated preparation of arylsulfatase A into a suitable container and freeze-drying the sample.

12. A process according to claim 11, further comprising an initial step I) of concentrating the arylsulfatase A by tangential flow filtration.

13. A process according to any of claims 11 or 12, wherein the chromatography column used in step II of the purification process is an anion exchange column.

14. A process according to claim 13, wherein said anion exchange column is a DEAE Sepharose column or a DEAE Streamline column.

15. A process according to any of claims 11 to 14, wherein the chromatography column used in step III of the purification process is a hydrophobic interaction column.

16. A process according to any of claims 11 to 15, wherein purification of the sample in step IV of the purification process is accomplished by tangential flow filtration.

17. A process according to any of claims 10-16, wherein said cation chromatography membrane or resin and said anion exchange membrane or resin used in the polishing step are connected in a series.

18. A process according to any of claims 11 to 17, wherein the filtration of the sample as performed in step VI of the purification process is replaced by or combined with contacting the sample with a detergent, preferably prior to step V or preferably prior to step II of the purification process.

19. A formulation of arylsulfatase A, which is obtainable or obtained by a process according to any of claims 1-18.

20. A formulation according to claim 19 comprising at least 98% bioactive aryl sulfatase A as determined by reverse phase HPLC.

21. A formulation according to claim 19 or 20, wherein said aryl sulfatase A has a specific activity of at least 20 U/mg, preferably 50 U/mg.

22. A formulation according to claims 19 to 21, which does not comprise

a) a vehicle, such as a peptide or polypeptide, for delivery of aryl sulfatase A into the central nervous system, and

b) a component capable of causing opening or disruption of the blood brain barrier, and

c) an intact cell

23. A formulation comprising an effective amount of aryl sulfatase A, said formulation being obtainable by a process according to any of claims 1 to 18 for use as a medicament.

24. A formulation of aryl sulfatase A according to claim 23 for use as a medicament for reducing the sphingolipid 3-O-sulfogalgactosylceramide levels within cells in the peripheral nervous system and/or within the central nervous system in a subject suffering from and/or being diagnosed with metachromatic leukodystrophy.

25. Use of a formulation comprising an effective amount of arylsulfatase A for the manufacture of a medicament for reducing the levels of galactosyl sulphatide in cells within the central nervous system in a subject suffering from and/or being diagnosed with metachromatic leukodystrophy.

26. Use according to claim 25, wherein the effective amount of arylsulfatase A is such that:

a) effective levels of the enzyme are sustained in circulation for not less than 8 days, and/or

b) effective levels of the enzyme are sustained in visceral organs, sciatic nerve and brachial plexus for not less than 8 days, and/or

c) effective levels of the enzyme are sustained in the liver for not less than 8 days,

subsequent to intravenous administration of said formulation.

27. Use according to any of claims 25 or 26, wherein the effective amount of arylsulfatase A is such that:

a) effective levels of the enzyme are sustained in circulation, and/or

b) effective levels of the enzyme are sustained in visceral organs, sciatic nerve and brachial plexus, and/or

c) effective levels of the enzyme are sustained in the liver,

by repeated intravenous administration of the formulation on a weekly, bi-weekly or monthly basis.

28. Use according to any of claims 25-27, wherein said arylsulfatase A formulation is a formulation according to any of claims 19-24.

29. Use according to any of claims 25-28, wherein the effective amount corresponds to a dose of arylsulfatase A of between 5 and 100 mg enzyme per kg of body weight.

30. Use according to any of claims 25-29, wherein the arylsulfatase A has a specific activity of at least 50 U/mg.

31. Use according to any of claims 25-30, wherein said medicament is for administration to a subject which do not receive any additional medical treatment for reduction of the sphingolipid 3-O-sulfogalgactosylceramide levels, including:

a) administration a formulation comprising a vehicle, such as a peptide or polypeptide, for delivery of the enzyme (arylsulfatase A) into the central nervous system, and

b) administration of a formulation capable of causing opening or disruption of the blood brain barrier, and

c) administration of an intact cell.

32. A method of treating/alleviating a symptom of a disorder associated with increased lysosomal storage of sphingolipid 3-O-sulfogalgactosylceramide, said method comprising administering to a subject a formulation of arylsulfatase A obtained or obtainable by a process according to any of claims 1 - 18 and thereby obtaining a reduction in the galactosyl sulphatide levels in cells within said subject.

33. A method according to claim 32, wherein said cells are cells within the peripheral nervous system and/or cells within the central nervous system.

34. A method according to claim 33, said method comprising administering said formulation of arylsulfatase A by a route other than intracerebroventricular, spinal, intrathecal or intracranial administration.

35. A method according to any of claims 32-34, wherein said aryl sulfatase A formulation is a formulation according to any of claims 19-24.